Full Length Research Paper

Increased COX-2 expression in patients with ovarian cancer

Yang Lin[1], Manhua Cui[1], Yanyu Shi[2], Fengwen Wang[1], Quyuan Wang[1]* and Hong Teng[1]

[1]Department of Gynaecology and Obstetrics, the Second Hospital of Jilin University, China.
[2]Jilin Province Product Quality Supervision Test Institute, Changchun 130022, China.

The pathogenesis of ovarian cancer remains poorly understood. Genome-wide gene expression profiling can provide novel genetic data involved in the pathogenesis of disease. In this study, using normal ovarian surface epithelium and abnormal ovarian surface epithelium of patients with ovarian cancer as models for cDNA microarray analysis, we found that cyclooxygenase-2(COX-2) expression of patients with ovarian cancer was increased. This result was further confirmed by semi-quantitative RT-PCR and Western blot. It was found that COX-2 was significantly up-regulated in ovarian cancer group in comparison with normal group on mRNA level. On protein level, COX-2 was also highly increased in ovarian cancer group. This study provides novel candidate molecules and suggests a potential local role for COX-2 as mediators of ovarian cancer and as markers of disease activity.

Key words: Ovarian cancer, COX-2, cDNA microarray, semi-quantitative RT- PCR, western blot.

INTRODUCTION

Ovarian cancer is a morphological and biological heterogenous disease (Vander et al., 1995; Carey et al., 1993). The most common subtypes are serous and endometrioid, accounting for about 50 and 10 to 20%, respectively, of all malignant ovarian neoplasms, and less common are the mucinous (5 to 10%) and clear cell (5 to 10%) subtypes (Kristensen et al., 2003; Green et al., 1999). The disease is currently thought to arise from the ovarian surface epithelium (OSE). Risk factors include increasing age, estrogen replacement therapy, a family history of ovarian and/or breast cancer, and nulliparity, whereas the oral contraceptive pill (OCP) decreases risk (Crijns et al., 2003; Hall et al., 2004). However, the cause and molecular mechanisms of ovarian cancer remain unclear. It is most likely that most of the molecular changes still need to be elucidated due to ovarian cancer complexity of the genome. Moreover, there is still a need for prognostic markers in this devastating cancer disease.

cDNA microarray technology is a burgeoning molecular biology technology over recent years, which can provide access to enormous genetic data sets with opportunities to discover novel disease mechanisms. cDNA microarray technique has unexampled advantages in disease study with the characteristics of high-flux and high efficiency. This approach has been applied to diseases of unknown cause to create new hypotheses relating to disease pathogenesis (Calvano et al., 2005; Ergun et al., 2007) and is shown to have prognostic (Korkola et al., 2007) and diagnostic applications (Takahashi et al., 2005; Aldred et al., 2004). Using the global genomic approach, we sought to identify novel gene transcripts engaging in common biological processes operating at the tissue level in ovarian cancer. Therefore, gene array were utilized in this study to detect corresponding gene transcripts on ovarian cancer.

MATERIALS AND METHODS

Study population and tissue

Ten women patients with ovarian cancer aged 23 to 55 were recruited from the Department of Cancer Diseases, the First Affiliated Hospital of Jilin University. The diagnosis of all patients was confirmed to be ovarian cancer on the basis of clinical, imaging and histopathological findings. In all cases, the diagnosis was made from a biopsy obtained from the ovarian surface epithelium. Disease-free ovarian surface epithelium were obtained during surgical ovarian or in the immediate postmortem period from

---

*Corresponding author. E-mail: wquyuan@yahoo.cn. Tel: 86-0431-8975634. Fax: 86-0431-897563.

patients who had submitted for organ donation for the purposes of medical research. Informed written consent was obtained from all subjects, and the study was approved by the Ethics Committee of Jilin University, China.

RNA extraction

Total RNA was isolated from ovarian surface epithelium (OSE) using TRIzol reagent (Invitrogen, USA) according to the manufacturer's protocol and as described in the online supplement. In fact, OSE was incubated with 1 ml TRIzol for 5 min at room temperature (RT). Cell debris was removed by centrifugation (12,000 × g at 4°C for 15 min) and 0.4 ml chloroform was added. The mixture was incubated for 15 min at RT after vortexing. The phases were separated by centrifugation (12,000 × g at 4°C for 15 min) and the aqueous phase was transferred to a new tube. 0.6× volume of isopropyl alcohol and a 0.1× volume of 3 M sodium acetate were added to this aqueous phase and incubated for 10 min at 4°C. The precipitated RNA was pelleted by centrifugation (12,000 ×g at 4°C for 15 min) and after the removal of the supernatant, the RNA was washed twice with 70% ethanol. After drying, the RNA was resuspended in 30 µl DEPC-treated water. The quality and quantity of the RNA was verified by the presence of two discrete electropherogram peaks corresponding to the 28S and 18S rRNA at a ratio approaching 2:1.

cDNA microarray

Isolation and validation of high-quality OSE mRNA from the ovarian cancer and the normal OSE mRNA were reversely transcribed to cDNAs, then, cDNAs were labeled with the directly incorporated fluorescently dUTP (cy-5 or cy-3) to prepare the hybridization probes. The mixed probes were hybridized to the cDNA microarray. After high-stringent washing, the cDNA microarray was scanned using the fluorescent signals and showed differences between two tissues by means of statistical analysis program.

Semi-quantitative RT-PCR

Using mRNA as template, single-stranded cDNAs were generated by Superscript II reverse transcriptase (Takara) according to the manufacturer's directions. The COX-2 primer sequences were as follows: sense prime: 5′-GAACAACATTCCCTTCCTTCG-3′; anti-sense prime: 5′-GAAGTTCCT TAT TTCCTTTCACACC-3. GAPDH (Applied Biosystems) served as the internal control. The PCR conditions were 94°C for 3 min, followed by 30 cycles of DNA amplification (45 s at 94°C, 1 min at 61°C and 90s at 72°C) and 5 min incubation at 72°C. PCR products were separated by electrophoresis at a constant voltage (2 V/ cm) in a 1.0% (w/v) agarose gel. Images were captured using a Gel Print 2000i/VGA (Bio Image), and the integrated densities value (IDV) was analyzed with computerized image analysis system (Motic Images Advanced 3.2). All DNA manipulations were performed as described by Sambrook et al. (2001).

Western blot

In order to detect protein expression level of COX-2, western blot was performed as described in the online supplement. Protein homogenates of OSE samples were prepared by rapid homogenization in 10 volumes of lysis buffer (2 mM EDTA, 10 mM EGTA, 0.4% NaF, 20 mM Tris-HCl, pH7.5). OSE homogenate were centrifuged at 17,000 g for 1 h at 4°C and the protein concentration in the supernatant was determined by the Coomassie (G250)

binding method. Equal amounts of protein (20 µg) from each sample were loaded and separated into a 7.5% gradient SDS-PAGE under denaturing conditions. Electroblotting proteins were transferred onto nitrocellulose membranes. After blocking with 5% BSA overnight at 4°C, membranes were incubated for 2 h at room temperature in agitation with the following antibodies: Rabbit polyclonal anti- COX-2 (dilution 1:500; Santa Cruz Biotechnology, USA) and rabbit polyclonal anti-β-actin (dilution 1:100; Santa Cruz Biotechnology, USA). Secondary horseradish peroxidase conjugated rabbit anti-goat / goat anti-rabbit antibodies (Santa Cruz Biotechnology, USA) were used at 1:3000 dilution for 2 h at room temperature in agitation. Immunoreactive bands were visualized using the enhanced chemiluminescence (ECL kit, Santa Cruz Biotechnology, USA) and scanned using Chemi Imager 5500 V2.03 software. The integrated densities value (IDV) was analyzed with computerized image analysis system (Fluor Chen 2.0) and normalized with that of β-actin.

Statistical analysis

To calculate the statistical differences between the control and ovarian cancer, the statistical package SPSS13.0 (SPSS Incorporated, Chicago) was used for all analysis.

RESULTS

COX-2 differentially expressed by cDNA microarray

Identifying functionally related genes that distinguished the patients with ovarian cancer and the control subjects was interesting. There were 204 differentially expressed transcripts genes in the patients with ovarian cancer when compared with the control subjects, 94 genes were up-regulated while 110 were down-regulated in the ovarian cancer. The gene list is available for review on the National Center for Biotechnology Information's Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/query). One of these genes was identified as COX-2, which was significantly up-regulated in ovarian cancer in comparison with the normal by cDNA microarray.

Semi-quantitative RT-PCR analysis of COX-2 expression

In order to detect the mRNA expression of COX-2 in patients with ovarian cancer, semi-quantitative RT-PCR was conducted. As shown in Figure 1, the expression of COX-2 was significantly higher in OSE with ovarian cancer than the normal OSE on mRNA level (P < 0.01). This result further confirms that COX-2 expression was up-regulated in patients with ovarian cancer in comparison with the normal women.

Western blot analysis of COX-2 expression

The western blot was performed to detect protein expression of COX-2. As shown in Figure 2, the expression of
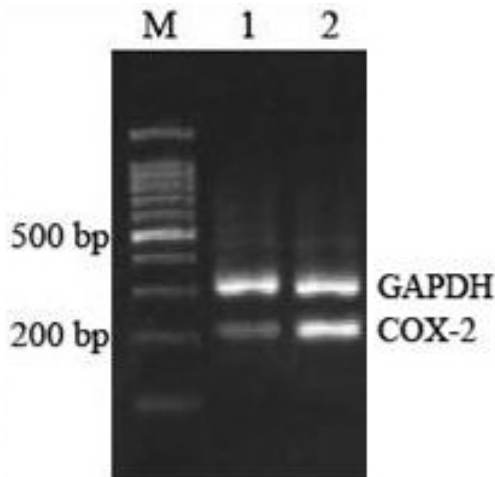
Figure 1. Semi-quantitative RT-PCR analysis of COX-2 mRNA expression in ovarian cancer. M, Marker; 1, normal OSE group; 2, OSE with ovarian cancer.
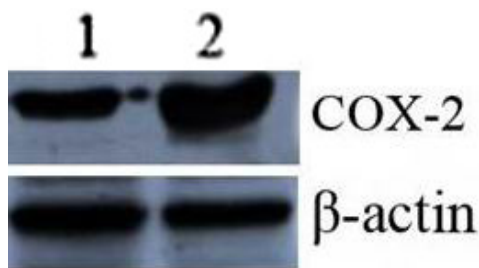

Figure 2. Weston-blot analysis of COX-2 protein expression in ovarian cancer. 1, Normal OSE group; 2, OSE with ovarian cancer.

COX-2 was significantly higher in OSE with ovarian cancer than the normal OSE on protein level (P < 0.01), which was compatible with mRNA expression of COX-2 in OSE with ovarian cancer.

DISCUSSION

In this study, cDNA microarray analysis was used to determine functionally related genes that may be expressed at different levels between OSE with ovarian cancer and controls; 94 genes were markedly up-regulated while 110 genes were downregulated in the ovarian cancer. COX-2, selected for further study, was up-regulated in the OSE by bioinformatics analysis. Semi-quantitative PCR verified that COX-2 increased on the mRNA level and furthermore, COX-2 also increased on the mRNA level by Weston-blot. This study provides preliminary data on the involvement of previously unidentified COX-2 protein in the pathogenesis of ovarian cancer.

COX-2 is an inducible isoform of cyclooxygenase. It was considered formerly that it played an important role in inflammation reaction. Furthermore, it has been shown to be upregulated in various carcinomas and to have a central role in tumorigenesis. It was reported that PGE_2 and 6-keto $PGF_{1\alpha}$ levels were elevated in colorectal cancers (Oka et al., 1994; Pugh and Thomas, 1994). The increase in prostaglandins may be explained by increased cyclooxygenase expression or increased cyclo-oxygenase catalytic activity. Eberhart and DuBois (1994) reported that COX-2, but not COX-1, was elevated in colorectal cancers. They found that approximately 50% of adenomas and 80 to 85% of adenocarcinomas had increased expression of COX-2, suggesting that COX-2 could be involved in colorectal carcinogenesis (Eberhart et al., 1994). These results were compatible with COX-2 expression which was up-regulated in ovarian cancer, which further confirmed that COX-2 play a key role in various carcinomas.

In conclusion, we have demonstrated that COX-2 was significantly up-regulated in patients with ovarian cancer in comparison with the normal human. This study provides a new approach for studying the mechanism underlying the pathogenesis of ovarian cancer. This suggests that increased COX-2 expression may be a potential target in the treatment of ovarian cancer.

REFERENCES

Aldred MA, Huang Y, Liyanarachchi S (2004). Papillary and follicular thyroid carcinomas show distinctly different microarray expression profiles and can be distinguished by a minimum of five genes. J. Clin. Oncol. 22: 3531–3539.

Calvano SE, Xiao W, Richards DR (2005). Inflammation and Host Response to Injury Large Scale Collaborative Research Program. A network-based analysis of systemic inflammation in humans. Nature, 437: 1032–1037.

Carey MS, Dembo AJ, Simm JE (1993). Testing the validity of a prognostic classification in patients with surgically optimal ovarian carcinoma; a 15 year review. Int. J. Gynecol. Cancer, 3: 24–35.

Crijns APG, Boezen HM, Schouten JP (2003). Prognostic factors in ovarian cancer: current evidence and future prospects. Eur. J. Cancer, 1(6): 127–145.

Eberhart CE, Coffey RJ, Radhika A, Giardiello FM, Ferrenbach S DuBois RN (1994). Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas.Gastroenterology. 107: 1183-1188.

Ergun A, Lawrence CA, Kohanski MA (2007). A network biology approach to prostate cancer. Mol. Syst. Biol. 3: 82-87.

Green JA, Berns E, Hensen-Logmans S, Vander Burg ME, Van Dam PA, Van Diest P (1999). On behalf of the EORTC Gynaecological Cancer Study Group. Biological markers in ovarian cancer – implications for clinical practice. CME. J. Gynaecol. Oncol. 4(1): 13–21.

Hall J, Paul P, Brown B (2004). Critical evaluation of p53 as a prognostic marker in ovarian cancer. Expert. Rev. Mol. Med. 6: 1–20.

Korkola JE, Baveri E, DeVries S (2007). Identification of a robust gene signature that predicts breast cancer outcome in independent data sets. BMC Cancer, 7: 61-69.

Kristensen GB, Kildal W, Abeler VM, Kaern J, Vergote I (2003). Large scale genomic instability predicts long-term outcome for women with invasive stage I ovarian cancer. Ann. Oncol. 14: 1494–500.

Oka M, Inaba A, Uchiyama T, Hazama S, Shimoda K, Suzuki M and Suzuki T (1994). Prostaglandin E2 levels and lymphocyte subsets in portal venous drainage of colorectal cancers. Am. J. Surg. 167: 264-267.

Vander Burg MEL, Van Lent M, Buyse M, Kobierska A, Colombo G, Favalli G (1995). The effect of debulking surgery after induction chemotherapy on the prognosis in advanced epithelial ovarian cancer. N. Engl. J. Med. 332: 629–634.